UNIVERSIDADE DE LISBOA

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Orientado por:

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Resumo

Enquadramento: Apesar dos avanços na área da transplantação de células progenitoras hematopoiéticas, o transplante alogénico continua a associar-se a importantes complicações, com elevada morbi-mortalidade, como a doença de enxerto-contra-hospedeiro crónica e neoplasias secundárias. A doença de enxerto-contra-hospedeiro crónica e o seu tratamento foram identificados como potenciais factores de risco para o desenvolvimento de neoplasias secundárias após transplante, nomeadamente neoplasias sólidas. No entanto existem poucos estudos que analisem o papel da doença de enxerto-contra-hospedeiro crónica no risco de neoplasias secundárias.

Objectivo: Analisar e clarificar o papel da doença de enxerto-contra-hospedeiro crónica e do tratamento imunosupressor associado, no desenvolvimento de neoplasias secundárias após transplante alogénico de células progenitoras hematopoieticas. Como objectivo final, este trabalho pretende aumentar a sensibilização para esta complicação tardia, no seguimento a longo-prazo destes doentes.

Método: Revisão sistemática da literatura, através da pesquisa na base de dados Pubmed, com inclusão final de 29 artigos científicos.

Resultados: A doença de enxerto-contra-hospedeiro crónica parece ser um factor de risco independente para neoplasias sólidas, nomeadamente para carcinomas pavimento-celulares de regiões frequentemente afectadas pela doença, como a cavidade oral, pele e, nalgumas populações, o esófago. O risco de neoplasia sólida parece aumentar com a duração do tratamento imunosupressor, quando este é superior a 24 meses, e com o uso de azatioprina. A associação da doença de enxerto-contra-hospedeiro crónica com outros tipos de neoplasias secundárias foi inconclusiva.

Conclusão: Os doentes com doença de enxerto-contra-hospedeiro crónica parecem ter um risco aumentado para neoplasias secundárias, nomeadamente neoplasias sólidas. Estas neoplasias tendem a surgir tardiamente, o que sugere um benefício na instituição de rotinas de rastreio oncológico a longo-termo, sobretudo para neoplasias da pele, cavidade oral, e nalgumas populações, esófago.

Palavras-Chave: Segundas neoplasias, doença de enxerto-contra-hospedeiro crónica, transplante alogénico de células progenitoras hematopoiéticas, neoplasias sólidas.

O Trabalho Final exprime a opinião do autor e não da FML.

Abstract

Background: Despite the advances in hematopoietic stem cell transplantation, allogeneic transplants are still associated with significant morbidity and mortality, due to late complications, such as chronic graft-versus-host disease and secondary malignancies. Both chronic graft-versus-host disease and its treatment have been implied as potential risk factors for secondary malignancies in patients undergoing allogeneic hematopoietic stem cell transplant, particularly for solid tumors. However the literature on the role of chronic graft-versus-host disease on the development of secondary malignancy is scarce.

Goals: Analyze and clarify the role of chronic graft-versus-host disease, and its immunosuppressive treatment, on the development of secondary malignancies after allogeneic stem cell transplantation. The final goal is to increase awareness for this late complication in the long-term follow up of these patients.

Methods: This is a systematic literature review, covering 29 articles, after a thorough search in Pubmed database.

Results: Chronic graft-versus-host disease seems to be an independent risk factor for solid tumors, namely for squamous cell carcinomas of regions frequently affected by the disease, such as the oral cavity, skin and, in some populations, the esophagus. The risk for solid tumors seems to increase with treatment duration, when surpassing 24 months, and with the use of azathioprine. The association between chronic graft-versus-host disease and other secondary malignancies was inconclusive.

Conclusions: Patients with chronic graft-versus-host disease seem to have an increased risk for secondary malignancies, namely solid tumors. Solid tumors tend to occur later in the follow-up, suggesting a benefit in cancer screening guidelines for the long-term-survivors, particularly for skin, oral cavity, and esophagus cancer.

Key-Words: Secondary malignancies, chronic graft-versus-host disease, allogeneic hematopoietic stem cell transplant, solid cancers.

List of Abbreviations

aGVHD – Acute Graft-versus-Host Disease
AML – Acute Myeloid Leukemia
allo-HSCT – Allogeneic Hematopoietic Stem Cell Transplantation
ATG – Anti-Thymocyte Globulin
BCC – Basal Cell Carcinoma
cGVHD – Chronic Graft-versus-Host Disease
HSCT – Hematopoietic Stem Cell Transplantation
HL – Hodgkin's Lymphoma
IS – Immunosuppressive Treatment
MMF – Mycophenolate Mofetil
MDS – Myelodysplastic Syndrome
PBSC – Peripheral Blood Stem Cells
PTLD – Post-Transplant Lymphoproliferative Diseases
Treg – Regulatory T-Cells
RR – Relative Risk
SCC – Squamous Cell Carcinoma
ST – Solid Tumor
SIR – Standard Incidence Ratio
TRM – Transplant-Related Mortality

Table of Contents

Introduction

Hematopoietic stem cell transplantation (HSCT) is the standard of care for many hematologic malignancies, and some congenital or acquired disorders of the hematopoietic system(1). Since the first transplant procedure, 50 years ago, HSCT utilization expanded all over the world, being effectively applied in solid tumors, congenital immunodeficiency disorders, and hereditary metabolic diseases(1).

The dramatic increase in the number of HSCT, namely allogeneic transplants (allo-HSCT), was possible due to the expansion to unrelated and alternative donors and the development of reduced intensity-conditioning regimes. These procedures, together with advances in the post-transplant supportive care have led to a decline in transplant-related mortality (TRM), and consequently an increase in the number of long-term survivors(2). Despite this improvement, allo-HSCT is still associated with an important morbidity and long-term mortality, with fifteen-year survivors having mortality rates at least twice as high as the general population(3).

According to the literature, the leading causes of mortality after transplant are secondary malignancies, disease relapse, infections, chronic graft-versus-host disease (cGVHD) and respiratory and cardiovascular diseases(4).

Focusing on secondary malignancies, these include lymphoproliferative diseases (PTLD), secondary leukemia, and solid tumors. Both cGVHD and its treatment have been reported as potential risk factors for the development of certain types of secondary malignancies, namely solid tumors(5–8); however there is still no consensus regarding the mechanisms through which cGVHD can increase this risk.

The main purpose of this review is to analyze and clarify the suggested role of cGVHD in the development of secondary malignancies, with the ultimate goal of increasing the awareness of this complication in the long-term follow-up of patients undergoing allo-HSCT. Furthermore, we aim to evaluate the impact of the type and duration of immunosuppressive treatment (IS) for cGVHD in the development of secondary malignancies.

As such we begin by providing an overview of cGVHD and the plausible mechanisms responsible for the suggested association between cGVHD and secondary malignancies. We then proceed with a systematic review of the literature, aiming to find evidence regarding this association.

Chronic Graft-versus-Host Disease - Overview

cGVHD is a major late complication of allo-HSCT, affecting 30 to 70% of transplanted patients(9,10). Its prevalence has been increasing, possibly due to factors such as the increasing age of recipients, use of unrelated-donors and peripheral-blood progenitor cells, and the advances in supportive care, which increase long-term HSCT survival rates(11).

cGVHD is a multisystem disorder with features of auto-immunity, that stems from the interaction between the donor's and the host's immune system. Despite the complex pathophysiology of cGVHD, studies consistently show an immune-deregulation of several populations of T and B-cells and an abnormal production of cytokines and chemokines, which contribute to the amplification of the inflammatory response and tissue damage. The resulting chronic immune-dysfunction and the subsequent long-term IS contribute to significant mortality (12).

Both infused donor T-cells and engrafting donor T-cells seem to have a role in the development of cGVHD. The studies suggesting a role for the infused donor T-cells show a lower incidence of cGVHD in T-cell depleted grafts and after anti-thymocyte globulin (ATG) therapy(13). The studies suggesting a role for the engrafting donor T-cells show an imbalance between CD4+ regulatory T-cells (Treg), which are decreased, and effector cells such as CD4+ conventional T-cells and CD8+ cells, that mediate the disease directly or through the production of inflammatory cytokines.

Within the B-cell compartment, studies have shown an increase in BAFF levels, and a subsequent increase in B-cells' half-life with further differentiation into plasma cells. These latter will generate auto and allo-antibodies, which seem to correlate with the onset and severity of cGVHD(9).

The presentation of cGVHD is variable, there being cases of single organ involvement and cases affecting several sites, including the skin, oral mucosa, digestive tract, liver and eyes. Certain manifestations are considered diagnostic, such as poikiloderma; lichen-like lesions of the skin, genitals and oral cavity; skin sclerosis; superior esophageal stenosis; esophageal webbing; bronchiolitis obliterans and connective tissue alterations (fasciitis, sclerosis and articular stiffness/contracture). Frequently cGVHD displays distinctive manifestations, which are suggestive but not diagnostic; these

correspond to autoimmune features such as malar rash, sicca syndrome, arthritis or cholestasis. The diagnosis of cGVHD requires the presence of one diagnostic manifestation or at least one distinctive clinical sign confirmed by biopsy or other relevant tests.

Typically the disease presents within the first year post-transplant, although it can appear later on. Occasionally it is preceded by acute GVHD (aGVHD).

Formerly the classification of acute or chronic GVHD was exclusively based on the time of onset, with cGVHD occurring more than 100 days post-transplant. However, in 2005 the NIH GVHD Consensus Response Criteria Working Group created criteria for a standardized diagnosis based on clinical features(14). Apart from the classic aGVHD there is a late form with characteristics of aGVHD that presents/persists after 100 days; besides the classic cGVHD there is the so-called overlap syndrome, defined by the coexistence of aGVHD and cGVHD features. This working group also proposed a scoring system for organ-specific (0-3) and global (mild, moderate, severe) severity, replacing the classic "limited" and "extensive" classifications. These modifications were maintained after a subsequent review in 2014, which addresses controversies as overlap syndrome and active disease versus past tissue damage(10).

The complex biology of cGVHD justifies the lack of available successful strategies to prevent and treat this disease. While for the prevention of aGVHD there is an immune-suppressive regimen that is started soon after transplant to control the infused donor T-cell activation, usually combining methotrexate with a calcineurin inhibitor (cyclosporine/tacrolimus)(15), for cGVHD there are few successful strategies. The use of marrow harvested stem cells instead of peripheral blood stem cells (PBSC)(16), and the in-vivo T-cell depletion with ATG, in the conditioning regimen, seem to decrease the incidence of cGVHD(17,18).

Regarding the treatment of cGVHD, the available options suppress the inflammatory response, being efficacious in managing many of the clinical manifestations(15). The first line treatment for cGVHD is corticosteroids in combination with a calcineurin inhibitor, such as cyclosporine or tacrolimus. Although this dual treatment allows the use of a lower steroid dose, and a lower induced toxicity, it did not demonstrate any benefit on the mortality rate(19,20). Systemic treatment, lasting at least 1 year, is

recommended for patients with moderate to severe disease(21); mild disease can often be treated topically, with efficacy.

However, 50% of patients fail to respond to the frontline treatment. We consider failure when there is clinical progression under high doses of prednisone (1mg/Kg/d) for at least 2 weeks, clinical stability only under high doses of prednisone (>0.5mg/Kg/d) for 1-2 months, or when it's not possible to taper prednisone to lower doses (<0.5 mg/kg/d)(22). So far there is no established second-line treatment for refractory cGVHD patients, as such it is recommended, if possible, to enroll these patients in clinical trials. Available second-line drugs include(21,23):

- immune suppressive nonspecific agents, that spare Tregs, such as: m-TOR inhibitors (sirolimus), pentostatin for skin involvement, and Mycophenolate mofetil (MMF);
- monoclonal antibodies, that cause B cell depletion, such as rituximab;
- specific pathway inhibitors, such as: ibrutinib (recently approved), imatinib, ruxolitinib; NF-kb and proteasome inhibitor bortezomib;
- immune regulatory treatments, which induce tolerance by inducing Treg expansion, such as extracorporeal photophoresis for skin or oral cGVHD, IL-2, therapy and Treg infusion.

Besides these approaches, cGVHD patients should receive supportive treatment, which may include analgesics, infectious prophylaxis, eye drops for dryness, among others.

Available studies suggest that the chronic inflammation and immune dysregulation of cGVHD may cause malignant transformation. The impact of cGVHD and/or it's IS treatment on the development of secondary malignancies remains uncertain. However, it is possible that the disease's inflammatory context combined with the chronic immunosuppression contribute to failures in immune surveillance/vigilance and failures in tissue repair mechanisms, increasing the risk for tumoral evolution (24–26).

Secondary Malignancies after HSCT – Overview

Secondary malignancies are a known complication in long-term allo-HSCT survivors. A population-based Australian cohort (≥15 years follow-up) of 3273 allo-HSCT patients reported a cumulative incidence of secondary malignancies of 3.35% at 10 years, with

transplant recipients having at least twice the risk of secondary malignancies compared to the general population(5).

These malignancies can be grouped into three categories, which are: donor-type secondary leukemia (AML)/myelodysplastic syndrome (MDS), post-transplant lymphoproliferative disorders (PTLD), and solid tumors. While the first two tend to develop in the first 10 years post-transplant, the incidence of solid tumors continues to increase even 20 years after transplant(27). A study of 2150 allo-HSCT recipients showed that the cumulative incidence of secondary malignancies was 9.9% at 10 years, with PTLD plateauing at 4 years (1.6%), AML/MDS plateauing at 9 years (2.1%), and solid tumors increasing continuously, even after 13 years (5.6%)(6).

Focusing on solid malignancies, Rizzo studied 28874 allo-HSCT recipients and reported an incidence of solid cancer around twice the reported in the general population, reaching cumulative incidences of 2.5%, 5.8% and 8.8%, at 10, 15 and 20 years, respectively, with increased risk for cancers of the oral cavity, skin, liver, central nervous system, thyroid, bone, soft tissues, and melanoma(28). This distribution was confirmed by another study, reporting cumulative incidences of solid cancer of 0.7%, 2.2% and 6.7%, at 5, 10 and 15 years, respectively(29).

Many factors have been suggested to increase the risk for solid cancer after allo-HSCT, such as age at the time of transplant, with elderly patients having an increased risk due to age-related risk; pre-transplant treatment or conditioning regimen, with an increased mutagenic risk associated to irradiation; cGVHD and prolonged immunosuppression. Chronic GVHD seems to be an independent risk factor for all solid cancers, namely for cancers of the oral cavity(30).

Methods

This systematic review of the literature includes articles that report the incidence and risk for secondary malignancies after allo-HSCT, and consider GVHD amongst their variables.

The research question and the eligibility criteria were defined using the PICOD method:

- **P (participants):** recipients of allo-HSCT.
- **I (intervention):** patients with cGVHD.
- **C (comparison):** allo-HSCT recipients without cGVHD.
- **O (outcomes):**
    - primary outcome: secondary malignancies;
    - secondary outcomes: type of malignancy, immunosuppressive drug(s) (IS) utilized, duration of IS..
- **D (design):** systematic review including retrospective cohort and case-control studies.

This study was performed respecting the different phases recommended by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), and represented in Figure 1:

1. Identification/Information sourcing: we used the PubMed database searching, with combinations of the following keywords: Second Cancers, GVHD, HSCT, Solid Cancers, PTLD. This resulted in 1054 results
2. Screening: in this phase we excluded review articles, case-reports and studies not concerning allo-HSCT (n=1029). As such only 25 articles were selected for eligibility.
3. Eligibility: Title and abstract selection excluded 3 studies where cGVHD was not a risk variable; 22 articles remained for full-test reading.
4. Included: After screening the references of the eligible articles, we added 13 studies, from which 7 were selected, leading to a total of 29 articles in the final analysis.

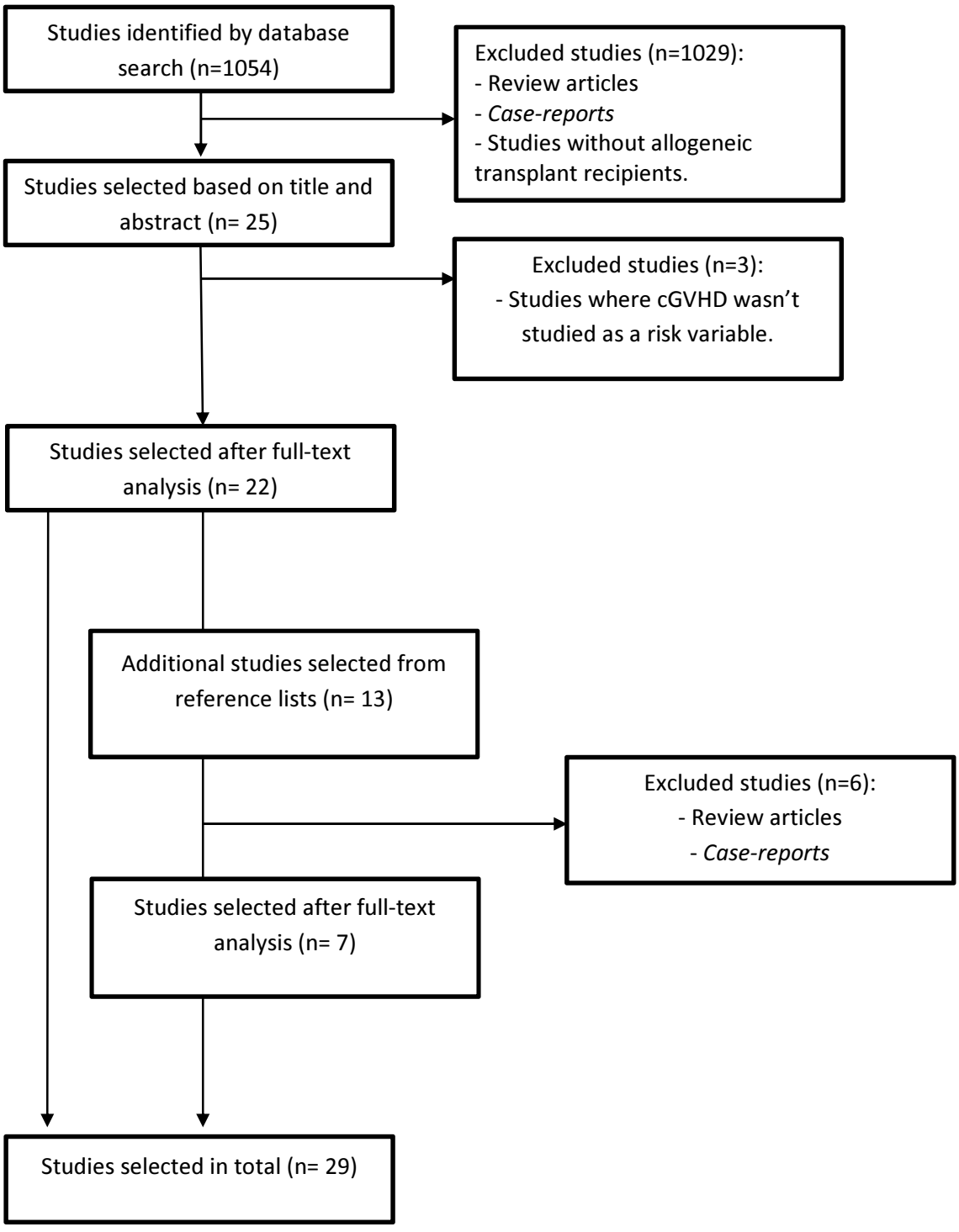

Figure 1 : Flow of study selection process.

Results and Discussion

This review includes a total of 29 articles published between 1996 and 2017 (period of 20 years). The characteristics of the studies can be consulted in supplementary Table 1.

Of the total 29 articles analyzed, 28 referred to retrospective cohort studies and 1 was a case-control study. These were conducted in different geographical locations, allowing for the collection of data from all five continents.

The studies were heterogeneous when it came to sample size, ranging from 38 to 68936 patients, with a median age at HSCT of 32 years (10-55 years). 21 studies included exclusively allo-HSCT recipients, of these 13 stated the source of progenitor stem cells, which were blood marrow, peripheral blood and cord blood in 88%, 9% and 3% of patients, respectively.

The incidence of cGVHD was stated in 22 studies, ranging from 10% to 97%, with an average incidence of 41%. The majority of studies didn't state the type of IS used; those that mentioned, mainly used corticosteroids, cyclosporine, azathioprine, MMF, ATG and tacrolimus. Only 1 study stated the duration of IS, with 24.7% doing more than 1 year.

Of the listed studies, 16 clearly stated the average time between HSCT and the development of secondary malignancies, with an average of 6,55 years (3,54-7,6 years) for all type secondary malignancies, 0,75 years for PTLD, 7 years for all-type secondary solid tumors (ST) (5,6-10 years) and 8,6 years (8,5-8,7 years) for thyroid cancer.

Impact of cGVHD on the development of secondary malignancies

From the 29 studies included in this analysis, 19 reported a statistically significant association between cGVHD and secondary malignancies, specifically ST, hematological malignancies or both.

Regarding the impact of cGVHD on secondary malignancies of all-type, this was only accessed in 3 studies(5). In Vajdic' study cGVHD patients had an increased incidence of secondary malignancies compared to the general population (SIR 1.51-2.82). The studies from Shimada and Abou-Mourad failed to demonstrate cGVHD as a risk factor for secondary malignancies; however in Shimada´s study 14 of the 16 cancer cases (BCCs or SCCs of the skin and oral cavity) were diagnosed in cGVHD patients (31)(32). Both the latter studies had a small sample size, which might explain the results.

cGVHD impact on Solid Tumors

From the 29 studies analyzed, 23 assessed the impact of cGVHD on the risk for secondary ST. On average ST were diagnosed between 1 to 10 years after allo-HSCT, with most cases appearing within a 5 to 8 year range.

Two studies focused on secondary thyroid cancers, with only one showing a significant correlation to cGVHD. In a study including 68936 HSCT recipients, 32 thyroid cancers were diagnosed, with cGVHD appearing as a significant risk factor(33). In a smaller study cGVHD failed to attain statistical significance as a risk factor, even though 6 of the 8 cases of thyroid cancers appeared in patients with cGVHD(34).

One study focused on cervical dysplasia, failing to demonstrate any association with genital cGVHD(35).

From the remaining 20 studies, 11 demonstrated an association between cGVHD and secondary ST, with a relative risk (RR) of 1.8 - 15.374, 9 were discordant with this association.

The studies that found association were unanimous in the increasing risk of oral carcinomas with cGVHD, namely squamous cell carcinomas (SCC). In the largest international study to date, Rizzo studied 28874 recipients of allo-HSCT and found cGVHD to be associated with an increased risk for oral and skin SCC(28). Majhail also reported cGVHD to be a risk factor for ST, particularly for oral carcinomas, with no statistically significant association with esophageal cancer (36). An international case-control study by Curtis found that the risk for SCC increased with the cGVHD grade, with severe cases having 10 times increased risk (37). However, when accounting for the type of IS, cGVHD didn't seem to be an independent risk factor for SCC, and this is probably due to the fact that severe cases are more likely to receive drugs such as azathioprine, which has demonstrated to increase the risk of cancer(38,39).

The studies that did not did association had a smaller sample size, and/or excluded patients who developed certain types of cancer, which might have modified the results. In the largest study 3372 allo-HSCT recipients were analysed, but patients with skin BCC and SCC were excluded (27). Other studies failed to demonstrate cGVHD as a risk factor for ST, even though the majority of tumors were diagnosed in sites previously affected by cGVHD. (6,40–45).

- Geographical variations in epidemiology of ST

Despite the general increase in the risk for ST with cGVHD, it is interesting to observe that certain types of ST are more prevalent in certain populations. This might be due to genetic and environmental factors or differences in cGVHD treatment.

Within the Asian studies, Japanese studies found association between cGVHD and oral or esophageal cancers. Atsuta reported in a cohort of 17545 allo-HSCT recipients, that extensive-type cGVHD increased the risk for esophageal (RR 5.3) and oral (RR 2.9)

cancers; whereas limited-type cGVHD was a significant risk factor for skin cancer (RR 5.8) (46). Yokota also demonstrated that cGVHD was an independent risk factor oral (RR 2.9) and esophageal (RR 4.9) SCC(47). Hasegawa found that all cases of oral and esophageal SCC were diagnosed in cGVHD patients(7).

In the biggest Taiwanese study to date, Chien analyzed a cohort of 2544 allo-HSCT recipients and reported cGVHD as a risk factor for oral and pharynx cancers(48). Another Taiwanese study has shown cGVHD to be a significant risk factor for all SCC, with 5 of 8 oral cancer-cases diagnosed in cGVHD patients(49).

American and European studies associate cGVHD to skin cancers. Curtis studied 19229 allo-HSCT recipients and reported a higher risk of oral (RR 5.1) and skin (RR 24.1) SCC in patients with cGVHD, with 9 of 14 oral SCC-cases and 7 of 8 skin SCC-cases developing in cGVHD patients(29). In a smaller cohort, Leisenring demonstrated that cGVHD was a highly significant risk factor for skin, mucosal SCC, and skin basal cell carcinomas(BCC) (50).

cGVHD impact on Hematological Malignancies

Only 2 studies focused on the risk of cGVHD for secondary hematological malignancies.

One study analyzed a cohort of 26901 allo-HSCT recipients and found that both aGVHD and cGVHD increased the risk for late-onset PTLD (appearing more than 1 year after transplant), with a RR of 3.17% for cGVHD(28).

The other study focused on the risk of secondary Hodgkin's Lymphoma (HL) in 18531 allo-HSCT recipients. This failed to demonstrate any significant association with cGVHD, even though all the HL cases (n=8) had grade II-IV aGVHD and/or cGVHD with more than 6 months of IS(51).

Impact of IS on secondary malignancies

Of the 29 analyzed studies, only 9 studied the impact of the IS, used for cGVHD, on secondary malignancies. Two treatment-related risk factors were identified, the type of IS and the duration of treatment.

As for the type of IS, the risk seems to increase in azathioprine-based regimes, although this might be due to an average longer duration of azathioprine-based regimes when compared with cyclosporine-based. The previously mentioned study by Curtis found that the risk for secondary malignancies was 18 times higher for regimens combining azathioprine, cyclosporine and steroids; this increased with addition of other drugs, PUVA or local field irradiation. In this same study azathioprine alone demonstrated to be a risk factor for skin SCC(37). In another study azathioprine alone proved to be a significant risk factor for the development of all-type ST (44). In a Taiwanese study azathioprine was as a significant risk factor for all-type ST, especially with cumulative doses above 15100 mg. In the same study there was a trend for an increased risk of ST

with sirolimus (p = 0.078) (48). According to an american study, patients treated with azathioprine plus cyclosporine, and to a lesser degree cyclosporine alone have an increased risk of late-onset PTLD(29).

As for treatment duration, Curtis showed that besides the severity of cGVHD, long durations of IS, especially those with azathioprine, was an important risk factor for secondary SCC(35); this risk increased after 24 months of combined treatment with azathioprine, cyclosporine and steroids. A cohort of 19229 transplant recipients also found an increased frequency of secondary malignancies in patients under IS for more than 2 years (30). In Turkey, Gündüz found that a duration of cGVHD above 1 year was associated with an increased risk of epithelial tumors (3.7% vs 0.1%), mostly head and neck tumors (39).

A study focusing on HPV-related cervical dysplasia reported cGVHD with 3 years IS, at least, as the only significant risk factor for secondary malignancies(50). However in another study focusing on PTLD, longer duration of IS don't seem to be a risk factor(29).

Limitations

The following limitations may have influenced the results of this study:

1. Review with lack of prospective studies and studies with larger sample size, limiting the attainment of statistical significance;
2. The majority of included studies were designed to analyze the development of secondary malignancies after HSCT, and not specifically the role of cGVHD;
3. The majority of included studies focused one a specific type of cancer, potentially sub-diagnosing other malignancies;
4. The difficulty in separating the effect of IS treatment from the isolated effect of cGVHD on the development of secondary malignancies.

Conclusion

Secondary malignancies are a concerning long-term complication in allo-HSCT recipients, with several studies reporting an increased risk. However the specific role of cGVHD in this setting remains unclear, with few studies delving into this issue.

This systematic review analysis 29 studies in which the development of secondary malignancies after allo-HSCT and cGVHD is assessed, with 12 studies confirming cGVHD to be a risk factor for all-type or specific ST. The majority of ST diagnosed after allo-HSCT correspond to SCC of the oral cavity, skin, and in certain populations, the esophagus, which are organ sites frequently affected by cGVHD.

The exact mechanisms behind the apparent association between cGVHD and secondary malignancies are yet to be clarified, but we speculate that the persisting tissue damage,

and the immune deregulation of cGVHD, contributes to the survival and proliferation of genetically abnormal cells. Furthermore, these patients are exposed to long periods of IS, which might increase the risk for tumor evolution. Results from this analysis suggest that the risk for ST is increased by longer durations of IS, after 24 months, and by the use of certain drugs such as azathioprine, with carcinogenic properties.

It's relatively hard to draw conclusions about the role of cGVHD in increasing the risk for other types of malignancies, namely hematological malignancies, thyroid and cervical cancers, due to the lack of studies in this setting. Such studies are much needed to further determinate the role of cGVHD and its treatment on secondary cancer risk.

Given the increased risk for ST in the long-term follow-up of cGVHD patients, and the significant mortality related to cancer, it might be beneficial to implement specific long-term cancer screening routines in these patients, especially for skin, oral and in some populations, esophageal cancer.

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Supplemental Materials

Table 1 : Characteristics and results of included studies.

Reference	Study type; Dimension; Location.	Age at HSCT; Type of HSCT; SC source.	cGVHD incidence; cGVHD treatment.	Number of secondary malignancies; Type of secondary malignancies; Years until onset.	Impact of cGVHD on development of secondary malignancies.
(Abou-Mourad, Lau, Barnett, & al., 2010)	> Retrospective cohort; > n = 419; > Canada.	> 14-57 years (m: 39); > 100% allo. > 91.4% BM, 7.7% PB, 0.9% others.	> 53.1%; > NS.	> n = 20; > 18 ST, 2 NHL; > 0.12-17.3 years (m: 6);	No association (*p* not statistical significant).
(Ades, Mary, Robin, & al., 2004)	> Retrospective cohort; > n = 133; > France.	> NS; > 100% allo. > 100% BM;	> 49.6%; > NS.	> n = 11; > 11 ST; > NS.	No association (*p* not statistical significant).
(Atsuta, Suzuki, Yamashita, & al., 2014)	> Retrospective cohort; > n = 17545; > Japan.	> 16-85 years (m: 40); > 100% allo; > 68% BM, 20% PB, 12% CB.	> 41.2% at 2 years; > NS.	> n = 269; > 269 ST; > NS.	**Highly significant association between E-cGVHD and ST** (RR 1.8); **oral** (RR 2.9) **and esophageal** (RR 5.3) **cancers**. **Significant association between L-cGVHD and skin cancer** (RR 5.8; p = 0.016).
(Au, Chan, Pang, & al., 2004)	> Retrospective cohort; > n = 615; > China.	> 18-65 years (m: 35.5); > 100% allo; > NS.	> 27%; > NS.	> n = 18 > 9 ST, 5 PTLD, 4 MDS/AML; > NS.	No association (*p* not statistical significant).

(Baker K. , DeFor, Burns, & al., 2003)	> Retrospective cohort; > n = 3372; > USA.	> 1-67 years (m: 24); > 42% allo, 35% auto; > 100% BM;	> NS; > NS.	> n = 147; > 62 ST, 43 PTLD, 34 MDS/AML; > NS.	No association (*p* not statistical significant).
(Bhatia, Ramsay, Steinbuch, & al., 1996)	> Retrospective cohort; > n = 2150; > USA.	> 1-67 years (m: 20); > 65% allo, 35% auto; > 100% BM;	> NS; > NS.	> n = 54; > 22 PTLD, 17 ST, 11 MDS/AML, 2 NHL, 1 HL; > NS;	No association with SM or skin cancer (*p* not statistical significant).
(Cavalier, Shmalo, Yu, & al., 2006)	> Retrospective cohort/case-report; > n = 49; > USA.	> 17-66 years (m: 55); > 100% allo. > NS	> 47.2% (E); > NS.	> n = 18; > 18 skin cancers. > 0.17-2.17 years (m: 1);	No association with skin cancer (*p* not statistical significant).
(Chen, Chang, Li, & al., 2011)	> Retrospective cohort; > n = 170; > Taiwan.	> 15-60 years (m: 31); > 100% allo; > 80.6% BM, 19.4% PB;	> 48.2% (39.4% L, 8.8% E); > 24.7% treated for more than 1 year with Aza and steroids.	> n = 8; > 8 ST. > 5.2-20.8 years (m: 10);	**Significant association with ST** (RR 15.374, p = 0.004).
(Chien, Liu, Hong, & al., 2015)	> Retrospective cohort; > n = 2544; > Taiwan	> 20-45 years (m: 32); > 59.5% allo, 40.5% auto; > NS;	> 49.6%; > CsA (97.1%), ATG (25.1%), Tacrolimus (16.7%), Aza (16.7%), among others.	> n = 43; > 43 ST; > NS;	**Significant association with head and neck cancers** (RR 2.84; p = 0.046). Azathioprine increases significantly risk of SM (RR 2.55; p = 0.025), especially cumulative doses above 15,100 mg (RR 3.58; p = 0.01).
(Cohen,	> Retrospective	> 1.7-51.3 years	> 10%	> n = 32	**Significant association**

Rovelli, Merlo, & al., 2007)	cohort; > n = 68936; > EU.	(m: 11.2 – only considering the SM cases); > 49% auto, 42% allo; > NS;	> NS;	> 32 thyroid cancers; > NS (m: 6.2).	**with thyroid cancer** (RR 2.94; p = 0.017).
(Cohen, Rovelli, Van Lint, & al., 2001)	> Retrospective cohort; > n = 113; > Italy.	> 1.7-18 years (m: 10); > 100% allo; > NS;	> 60% (43% L, 17% E). > NS;	> n = 8 > 8 thyroid cancers; > 3.1-15.7 years (m: 8.5).	No association with thyroid cancer.
(Curtis, Metayer, Rizzo, & al., 2005)	> Case-control; > n = 24011; > International.	> 3.5-61.3 years (m: 26.5); > 100% allo; > NS;	> 72.4% in case-patients, and 51.6% in control-patients; > Case-patients regimens included CsA + Aza + Steroids + other (21.4%), CsA + Aza + Steroids (16.7%), Aza + Steroids (16.7%), and others (33.3%). – while 11.9% received no treatment. In control patients regimens included CsA + Steroids	> n = 183; > 183 ST; > 0.9-22.9 years (m: 7);	**Significant association with SCC**, when not considering the type of drug therapy or duration of treatment (RR 2.79; p = 0.01); subsequent models found a highly significant SCC risk for cGVHD IS treatment ≥ 24 months (RR 8.44) – in this model cGVHD was not found a significant independent risk factor for SCC. When looking at the type and duration of cGVHD treatment, the risk was highly significant for combined treatments with Aza, CsA and steroids (RR 18.61), especially for ≥ 12 months (38.71); and significant for Aza and steroids (RR 2.77; p = 0.07), especially for ≥ 24 months (RR 5.14; p = 0.09). When looking at cGVHD severity, SCC

			(22.5%), Aza + Steroids (15%), Steroids (12.5%), and others (37.5%) – while 12.5% received no treatment.		risk was highly significant in severe disease (RR 9.93) and significant in moderate disease (RR 2.73; p = 0.08). cGVHD was most strongly associated with skin SCC (RR 14.46; 95% CI = 1.84-113.3).
(Curtis, Rowlings, Deeg, & al., 1997)	> Retrospective cohort; > n = 19229; > USA.	> NS (m :25.5); > 97.2% allo, 2.8% syn; > 100% BM;	> 17%; > NS.	> n = 80; > 80 ST; > NS;	**Significant association with skin SCC** (RR 24.1; p < 0.001) **and oral SCC** (RR 5.1, p = 0.004).
(Deeg, Leisenring, Storb, & al., 1998)	> Retrospective cohort; > n = 212; > USA.	> 1-42 years (m:18); > 99% allo, 1% syn; > NS.	> 40.5%; > Patients treated with steroids, CsA, among others.	> n = 11; > 11 ST; > NS.	No association with SM (*p* not statistical significant).
(Deeg H. , Socié, Schoch, & al., 1996)	> Retrospective cohort; > n = 700; > International.	> 1.8-67 years (m: 18); > 100% allo; > 100% BM.	> 31%; > NS.	> n = 23; > 18 ST, 3 PTLD, 2 ALL; > NS (m: 7.58 for all cancers; m: 8.25 for solid tumors).	**Significant association with SM in the univariate analysis** (RR 3.7; p = 0.0099). In multivariate analysis treatments with Aza increased significantly the risk (RR 11.7; p < 0.001)..
(Gallagher & Forrest, 2007)	> Retrospective cohort; > n = 926; > Canada.	> 12-65 years (m: 39); > 100% allo; > 87% BM, 12% PB, 1% others.	> 74%; > NS.	> n = 30; > 30 ST; > NS (m: 6.8).	No association with SM (*p* not statistical significant).
(Gündüz, Özen, Şahin,	> Retrospective cohort;	> 5-71 years (m: 31);	> 41%; > NS.	> n = 15; > 12 ST, 3	**Significant association between cGVHD ≥ 1**

& al., 2017)	> n = 979; > Turkey.	> 100% allo; > 70% PB, 27% BM, 2% CB.		PTLD; > 0.25-1.67 years (0.75) for PTLD; 0.5-26.33 years (m: 7.75) for ST.	**year and SM** (RR 7.1; p = 0.001); these patients also had more epithelial cancer risk than ones with shorter disease duration (3.7% vs. 0.1%; p < 0.001).
(Hasegawa, Pond, Riftkind, & al., 2005)	> Retrospective cohort; > n = 809; > Japan.	> 15-70 years (m: 34); > 73.1% allo, 26.1% auto, 7% syn; > NS.	> 32.5% (13.1% L, 19.4% E); > NS.	> n = 19; > 19 ST; > 1-11.58 years.	**Significant association between E-cGVHD and ST** (RR 2.9; p= 0.0352).
(Landgren, Gilbert, Rizzo, & al., 2009)	> Retrospective cohort; > n = 26901; > USA.	> 0.1-68 years (m: 26.6); > 100% allo; > NS.	> 17%; > NS.	> n = 127; > 127 PTLD; > NS (83% developed within 1 year).	**Significant association with late-onset PTLD** (RR 3.17; 95% CI = 1.45-8.30) CsA (RR 3.18; 95% CI = 1.25-8.06) or CsA + Aza (RR 7.81; 95% CI = 1.18-51.94) increased risk for late-onset PTLD.
(Leisenring, Friedman, Flowers, & al., 2006)	> Retrospective cohort; > n = 4810; > USA.	> 0.3-72.6 years (m: 31.3); > 100% allo; > NS.	> NS; > NS.	> n = 253; > 253 ST; > NS (m: 7.9 for BCC, m: 6.3 for SCC).	**Significant association with SCC** (RR 3.0) **and skin BCC** (RR 1.6; p = 0.007).
(Lishner, Patterson, Kandel, & al., 1990)	> Retrospective cohort/case-report; > n = 56; > Canada	> NS; > 100% allo; > 100% BM.	> NS; > NS.	> n = 3; > 3 ST; > NS.	No association with SM (*p* not statistical significant).
(Majhail, Brazauskas, Rizzo, & al., 2011)	> Retrospective cohort; > n = 4318; > International	> 1-60 years (m: 29) in AML patients; 1-60 years (m: 36) in	> 32% (30-35%) at 3 years for AML patients; 46%	> n = 66; > 66 ST; > NS (m: 6).	**Significant association with ST** (RR 2.4; p = 0.001); **and oral cancer** (RR 12.7; p = 0.02); No

		CML patients; > 100% allo; > 84% BM, 16% PB.	(44-48%) at 3 years for CML patients; > NS.		association with esophageal cancer.
(Rizzo, Curtis, Socié, & al., 2009)	> Retrospective cohort; > n = 28874; > International	> 0.08-72.41 years (m: 27); > 100% allo; > 100% BM.	> 31% at 3 years; > NS.	> n = 189; > 189 ST; > NS.	**Significant association with skin and oral SCC** (RR 5.04; 95% CI = 2.90-9.00). The risk associated with cGVHD decreased in patients with acute leukemia for primary diagnosis. .
(Rowlings, Curtis, Passweg, & al., 1999)	> Retrospective cohort; > n = 18531; > USA	> 1-72 years (m: 26); > 100% allo; > NS.	> 30% (E); > NS.	> n = 8; > 8 HL; > 2.9-9.1 years (m: 4.2).	E-cGVHD was not found a statistically significant risk factor for HL.
(Savani, Stratton, Shenoy, & al., 2008)	> Retrospective cohort; > n = 38; > USA	> 9-60 years (m: 30); > 76% allo; > 24% PB.	> 97%; > 17% adult females did more than 3 months of IS.	> n = 15; > 12 SIL (HPV +), 3 ASCUS (HPV-); > NS (m: 4.25).	**Significant association between cGVHD requiring prolonged IS (≥ 3 years) and HPV-related cervical dysplasia** (OR 4.6; p = 0.019).
(Shimada, Yokozawa, Atsuta, & al, 2005)	> Retrospective cohort; > n = 557; > Canada	> 24.2-47.4 years (m: 35.8) for patients who developed a SM. 23-42.2 years (m: 32.6) for patients who did not; > 100% allo; > 100% BM.	> NS; > NS.	> n = 31; > 27 ST, 4 hematological malignancies; > NS (m: 6.79).	No association with SM (*p* not statistical significant).
(Vajdic,	> Retrospective	> 15.5-59.2	> 48%;	> n = 79;	**Significant association**

Mayson, Dodds, & al., 2016)	cohort; > n = 3273; > Australia	years (m: 40.4); > 100% allo; > 53.5% PB, 40.9% BM, 1.3% CB.	> NS.	> 76 ST. > 0.13-13.5 years (m: 3.54).	**with SM** (HR 1.65; p = 0.008) - cGVHD patients presenting a higher incidence of SM (SIR 2.06; 95% CI = 1.51-2.82).
(Yokota, Ozawa, Masanori, & al., 2011)	> Retrospective cohort; > n = 2062; > Japan	> 7-68 years (m: 36); > 100% allo; > 75.5% BM, 16% PB, 7.8% CB.	> 55.3%; > NS.	> n = 30; > 30 ST; > NS (m: 5.6).	**Significant association with ST** (RR 2.4; p = 0.043); **and oral and esophageal SCC** (RR 4.9; p = 0.019).

<u>Abreviations:</u> SC: stem-cell; allo, allogeneic-hematopoietic stem cell transplant; BM, bone marrow; PB, peripheral blood; NS, not stated; SM, secondary malignancy; ST, solid tumor; NHL, non-Hodgkin lymphoma; SM, secondary malignancy; CB, cord blood; E, extensive type; L, limited-type; HL, Hodgkin lymphoma; CsA, ciclosporine; ATG, anti-thymocyte globulin; auto, autologous-hematopoietic stem cell transplant; SCC, squamous cell carcinoma; Aza, azathioprine; IS, imunossupresive; syn, syngeneic; ALL, acute lymphoblastic leucemia; BCC, basocelular carcinoma; CML, chronic myeloid leucemia; SIL, squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; m, median; SIR, standardized indice ratio.